

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
23 October 2003 (23.10.2003)

PCT

(10) International Publication Number
WO 2003/087388 A3

(51) International Patent Classification⁷: **C12Q 1/68**,
C12P 19/34, C07H 21/04

(21) International Application Number:
PCT/US2002/020690

(22) International Filing Date: 28 June 2002 (28.06.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/302,909 3 July 2001 (03.07.2001) US

(71) Applicant (for all designated States except US): **THE BOARD OF TRUSTEES OF THE LELAND STANFORD UNIVERSITY** [US/US]; 900 Welch Road, Suite 350, Palo Alto, CA 94303 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **HASSIBI, Arjang** [CA/US]; 3352 Alma Street, Apt. 240, Palo Alto, CA 94306 (US). **POURMAND, Nader** [SE/US]; 23 Dover Court, San Carlos, CA 94070 (US).

(74) Agents: **MALLIE, Michael, J. et al.**; Blakely, Sokoloff, Taylor & Zafman LLP, 12400 Wilshire Boulevard, 7th floor, Los Angeles, CA 90025 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
4 March 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 2003/087388 A3

(54) Title: BIOLUMINESCENCE REGENERATIVE CYCLE (BRC) FOR NUCLEIC ACID QUANTIFICATION

(57) Abstract: The present invention concerns methods of quantifying nucleic acids using a bioluminescence regenerative cycle (BRC). In BRC, steady state levels of bioluminescence result from processes that produce pyrophosphate. Pyrophosphate reacts with APS in the presence of ATP sulfurylase to produce ATP. The ATP reacts with luciferin in a luciferase-catalyzed reaction, producing light and regenerating pyrophosphate. The pyrophosphate is recycled to produce ATP and the regenerative cycle continues. Because the kinetic properties of ATP sulfurylase are much faster than luciferase, a steady state results wherein concentrations of ATP and pyrophosphate and the rate of light production remain relatively constant. Photons are counted over a time interval to determine the number of target molecules present in the initial sample. The BRC process has a controllable dynamic range up to seven orders of magnitude and is sensitive enough to detect a few thousand molecules of target nucleic acid.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/20690

A. CLASSIFICATION OF SUBJECT MATTER		
IPC(7) : C12Q 1/68; C12P 19/34; C07H 21/04 US CL : 435/6, 91.2; 536/24.33		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) U.S. : 435/6, 91.2; 536/24.33		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Please See Continuation Sheet		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,274,320 B1 (ROTHBERG et al.) 14 August 2001 (14.08.2001), entire document, especially column 16, lines 7-67, column 17, lines 1-61.	1-35
Y	US 6,270,973 B1 (LEWIS et al) 07 August 2001 (07.08.2001) entire document, especially column 46, lines 19-67, column 47, lines 1-53.	1-35
Y,E	US 6,534,269 B2 (LIU et al) 18 March 2003 (18.03.2003), entire document, especially column 3, lines 35-67, column 4, lines 1-48.	1-35
Y	US 6,255,083 B1 (WILLIAMS) 03 July 2001 (03.07.2001), entire document.	1-35
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	
Date of the actual completion of the international search 17 November 2003 (17.11.2003)		Date of mailing of the international search report 04 DEC 2003
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450 Alexandria, Virginia 22313-1450 Facsimile No. (703)305-3230		Authorized officer Sūryaprabha Chundūru Telephone No. 703-308-0196

INTERNATIONAL SEARCH REPORT

PCT/US02/20690

Continuation of B. FIELDS SEARCHED Item 3:

Biosis, Embase, Medline, Lifesci, Caplus, East databases

search terms: multiple targets, pyrophosphate, sequencing, rolling circle